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I, SMILJA DRAGOSAVLJEVIC, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002951240 for a patent by ROYAL WOMEN'S HOSPITAL as filed on 23 August 2002.

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Third day of September 2003

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PRIORITY DOCUMENT

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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant:

ROYAL WOMEN'S HOSPITAL

Invention Title:

DEPLETION OF PLASMA PROTEINS

The invention is described in the following statement:

DEPLETION OF PLASMA PROTEINS

This invention relates to methods of analysis, and in particular to methods for the preliminary fractionation of samples in which low abundance molecules of interest, for example proteins, polysaccharides or fatty acids, are present together with more abundant molecules of little or no interest. The method is particularly applicable to samples of human biological fluids such as serum, plasma, tears, saliva, cerebrospinal fluid, uterine washings, amniotic fluid or urine. It is contemplated that the method of the invention will be especially useful for proteomic applications involving biomarker discovery. Products and kits for use in the method are also disclosed, and form part of the invention.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

Following the successful completion of the complete sequence of the human genome in the Human Genome Project, and corresponding successes with other genomes such as the mouse and the rat, there is an urgent need in the art to determine the function of the proteins which these genomes encode, and to determine how these proteins are expressed during various physiological states and in disease.

Proteomics is an area of research which seeks to define the function and relative expression profiles of subsets of proteins encoded by a given genome at a given time in a given cellular location. Proteomics separates, identifies, and characterizes the proteins expressed, retained, secreted or released by a cell or tissue in order to establish their function(s) and their potential relationship to the onset, type, stage and progression of diseases, as well as relapse and/or response to therapy.

One primary tool for protein separation and analysis of proteins is two-dimensional gel electrophoresis (2DE). Following separation by 2DE, proteins are characterized and identified, usually using matrix-assisted laser disorbtion interferometery (MALDI) peptide mass fingerprinting or other forms of advanced mass spectrometry, for example, electrospray mass spectroscopy (MS) or time-of-flight (TOF)/TOF MS, coupled to protein and genomic database searching.

Proteomics often encompasses the comparison of tissue samples from diseased and healthy people, in order to identify proteins whose expression is changed in disease. Proteins which are significantly altered in their expression, location or post-translational modification (PTM) in patients with a disease, compared to those in a group of healthy individuals, may represent protein targets for drug or discovery of biological markers, for example, endpoint and/or surrogate biomarkers. One application of proteomics is in the search for biological markers of disease onset, progression and treatment in elements of the blood, such as serum or plasma.

Unfortunately, the analysis by 2DE gels of proteins in samples of biological fluids such as serum and plasma is very difficult. This is because of the limited amount of protein able to be resolved by a gel, and the high dynamic range of proteins in many samples. These factors result in data obtained by 2DE from complex samples, such as unfractionated serum and plasma, being

dominated by the presence of proteins which are of high abundance in blood, for example, human serum albumin, immunoglobulin G (IgG), haptoglobin, fibrinogen, transferrin, α1-antiptrypsin, α2-macroglobulin, IgA, and IgM. Proteins with a concentration higher than 1mg/mL are generally considered to be of high abundance.

The presence of these abundant proteins severely limits the utility of 2DE in the investigation of low-abundance proteins such as cytokines, signal transduction proteins, hormonal mediators, and cancer biomarkers. The dynamic range problem is illustrated in Figure 1, which shows the results of 2DE of a sample of unfractionated human plasma. To date, one of the major hurdles in identifying biological markers in blood is the presence of very abundant proteins, such as albumin, which comprises more than 80% of the total protein present in plasma; see the circle in Figure 1. As the total amount of protein which can be loaded onto a gel is limited to less than approximately 120 mg, the maximum amount of "non-albumin" proteins that can be loaded is limited to approximately 36 mg, thus limiting the ability of this technique to visualize and identify putative clinically-relevant low abundance biomarker proteins. Rare proteins may be difficult if not impossible to detect. Similar, although less extreme, dynamic range problems are experienced with 2DE analyses of other non-blood biological samples such as urine, tissue extracts, and cell lysates.

One approach to solving this problem is to develop methods for removing albumin and other highly abundant proteins from blood samples such as serum and plasma before analysis, thus increasing the likelihood of identifying low abundance protein biological markers. In particular, a method to remove the 50 to 100 most abundant proteins from plasma before analysis is required.

We have now found that a surprisingly simple immuno-affinity procedure, combined with the use of existing solid phase affinity capture supports, can be

used to rapidly remove high abundance proteins from biological samples, giving a dramatic improvement in the separation of low-abundance proteins by 2DE.

It is to be clearly understood that while the invention is specifically illustrated with reference to proteins, a similar procedure may be used to separate any low-abundance molecule from a complex sample. In addition to low-abundance proteins, the procedure may also be used to separate low-abundance polysaccharides or fatty acids from a complex sample.

SUMMARY OF THE INVENTION

In a first aspect, the invention provides a method of depleting high-abundance molecules from a biological sample, comprising the steps of

a) subjecting the sample to affinity depletion using an affinity support with high affinity for a high abundance molecule, and/or

b) immunodepletion using an affinity support coupled to an antibody directed against whole or previously fractionated plasma or serum.

Preferably the sample is subjected to both affinity steps. While it is possible to perform the steps in either order, we have found that by performing step (a) before step b) much less antibody is required for substantially complete removal of high abundance molecules. Therefore this order is preferred.

Preferably the high abundance molecules are proteins, and the antibody is an avian antibody. Even more preferably, the high abundance protein is albumin.

Preferably the biological sample is a biological fluid such as serum, plasma, lymph, cerebrospinal fluid, amniotic fluid, cervicovaginal fluid, uterine fluid, or semen. Alternatively the sample may be conditioned medium from a cell or tissue culture, or may be a tissue extract,

especially an extract of a highly vascularized tissue.

The biological sample may be obtained from any mammalian species, including humans, companion animals such as dogs and cats, domestic animals such as horses, cattle and sheep, or zoo animals such as non-human primates, felids, canids, bovids, and ungulates. Preferably the sample is obtained from a human.

The mammal may be of either sex, may be of any age, and may be either healthy or suffering from any kind of pathological condition, including but not limited to infections, cancers, or chronic degenerative conditions. In other words, the method of the invention is applicable to any situation where it is desired to perform analysis in order to determine whether there is a change in the pattern of expression of a molecule.

The affinity support used in step (a) may be any such support which is known to have a high affinity for albumins, immunoglobulins or other highly abundant proteins, including but not limited to Cibacron blue F3GA affinity supports such as Affi-gel Blue (Bio-Rad Laboratories), Blue Sepharose (Amersham Biosciences), Protein A, Protein G or Protein A/G fusions. Other dye-ligands also that could be employed to remove abundant blood proteins include: Procion® Red HE3B, Reactive Blue MRB, Reactive Green H4G, Reactive Green HE4BD, Reactive Green HE4BD, Reactive Yellow M8G, and Reactive Brown M4R, all of which can be coupled to supports such as Sepharose 4B and 6B.

The affinity depletion in step (a) may involve the use of magnetic agarose beads (Dynabead M-280) as a solid phase matrix support for an affinity ligand for the magnetic separation of high abundance molecules from low abundance molecules.

Similarly, any solid-phase support which can be coupled to immunoglobulin to form an affinity support may be used in step (b); these include but are not limited to agarose gels such as Sepharose 4B or Sepharose 6B

(Pharmacia), cross-linked agarose, acrylamide-based and cellulose-based beads.

The antibody may be raised in any convenient bird or mammalian species. Where the antibody is an avian antibody, this may be raised in any convenient species of bird, but most conveniently will be raised in a poultry species such as a chicken, turkey, duck or goose. Most preferably the avian antibody is a chicken antibody. The high-abundance molecule may be conjugated to a carrier protein if necessary in order to increase immunogenicity.

The antibody used in step (b) may be a first generation polyclonal antibody raised against whole serum or plasma, or any fraction of these complex proteinaceous biofluid mixtures, suitably raised using an immunization schedule comprising multiple booster injections. More preferably the antibody is a second generation polyclonal antibody raised against plasma or serum that has already been subjected to at least one round of affinity depletion and immunoglobulination with IgY directed against homologous plasma or serum. The antibody may be produced and purified using any conventional method. Such methods are disclosed in US patents No. 5367054, No. 5420253, No. 4550019 and No. 4056737.

It is also to be clearly understood that the procedure may be performed prior to any separation technology. In addition to 2DE, other separation technologies include but are not limited to, one-dimensional gel electrophoresis (1DE), capillary electrophoresis, mass spectrometry, high-pressure liquid chromatography (HPLC), as-chromatography, or liquid chromatography.

In a second aspect, the invention provides a composition for immunodepletion of abundant molecules from a biological sample, comprising an antibody preparation directed against abundant molecules, coupled to a solid phase support. Preferably the antibody is a second generation avian polyclonal antibody, and the abundant

molecules are those in serum or plasma. Even more preferably the avian antibody is from chicken, and the serum or plasma molecules are serum or plasma proteins.

As in step (b) of the first aspect of the invention, the support may be any solid-phase support which may be coupled to immunoglobulin to form an affinity support.

In a third aspect the invention provides a device for the rapid processing of clinical samples in the method of the invention, comprising a generally cylindrical chamber having an opening at either end, in which each opening is adapted to fit sealingly to a receptacle, wherein sample can be transferred from one receptacle to the other via the chamber, and in which the chamber has transversely disposed within it multiple layers of an affinity support having high affinity for high abundance molecule(s), separated by a layer of an immuno-affinity support(s) coupled to one or more antibodies directed against abundant molecules. In a preferred embodiment the receptacle is a syringe barrel.

Preferably the high abundance molecule(s) is/are albumin and/or immunoglobulins, the antibody is avian, and the abundant molecule(s) is/are plasma or serum proteins.

The term "sealingly" means that the chamber fits to the receptacle sufficiently tightly that substantially no fluid can escape when fluid is passed from one receptacle to another via the chamber. The plane of each layer of the support is generally perpendicular to the axis of the chamber. In use, the chamber is connected at one end to a receptacle containing a fluid biological sample, and at the other end to an empty receptacle, and the sample is passed a number of times from one receptacle to the other through the chamber.

Preferably the receptacles are hypodermic syringes and the chamber is a Luer-type cartridge. More preferably both the chamber and the receptacles are made of plastics.

In a fourth aspect, the invention provides a kit for removal of high-abundance molecules from a biological sample, comprising:

a) a first affinity support with high affinity for high abundance molecules, such as albumin and/or other highly abundant proteins such as IgG; and

b) a second affinity support coupled to an antibody directed against whole sample or against abundant molecules in the sample.

Preferably the antibody is an avian antibody, and is directed against the whole serum or whole plasma, or against abundant serum or plasma proteins.

Preferably the kit also comprises a device according to the third aspect of the invention; optionally the kit may also comprise a diluent suitable for use with biological fluids.

In both the third and the fourth aspects of the invention the affinity supports are as described for the first aspect.

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

The term "high abundance protein" refers to a protein which is present at a concentration greater than 1mg/ml in a biological sample.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of 2DE of a sample of human plasma. The circle indicates spots representing albumin.

Figure 2A is a schematic illustration of the process for production of first and second generation polyclonal antibodies in chickens.

Figure 2B is a schematic illustration of the processes for affinity and immunodepletion of proteins

from human plasma.

Figure 3 is a Western blot of a 2DE of a sample of whole human plasma. The blot was probed with pooled first and second round chicken antibody raised against human plasma, and demonstrates the range of abundant protein antigens against which antibody responses have been mounted by the immunised chickens.

Figure 4 shows the results of 2DE of a sample of human plasma subjected to 4-7 IPG isoelectric focusing and 10% acrylamide SDS-PAGE (Criterion gel Bio-Rad), and visualized by SYPRO Ruby®. Figure 4A is a display of proteins present in unfractionated human plasma. Figure 4B is a display of proteins present after treatment of human plasma with Affi-gel Blue. Figure 4C is a display of proteins present after treatment of human plasma with Affi-gel blue and then anti-human plasma (AHP)-Sepharose 4B.

Figure 5 is a schematic representation of a cartridge device according to the invention for rapid processing of samples of biological fluids.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples and drawings.

Abbreviations used herein are as follows

AHP	anti-human plasma antibody
BPB	bromophenol blue
CHAPS	3-[(3-cholamidopropyl)-dimethylamino]-2-hydroxyl-1-propane
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
IgY	immunoglobulin Y
PEG	polyethyleneglycol
TBP	tributylphosphine

In a preferred embodiment of the invention, we have found that depletion of albumin using a Cibacron Blue-based affinity support greatly reduces the number of protein spots detectable by SYPRO Ruby® staining of 2DE gels. Using this step in conjunction with a second step of immunodepletion with an immuno-affinity support coupled to an IgY further reduces the number of spots, as well as enabling the detection of previously undetectable spots. The avian equivalent of IgG, usually referred to as IgY, is significantly different in its chemical and physical properties from IgG. In particular, in addition to having different amino acid composition and sequence, IgY has a much higher electrophoretic mobility than IgG, a much lower isoelectric pH, a higher molecular weight, and substantially different chemical stability. Under certain conditions IgY requires stabilization by non-ionic surfactants, whereas IgG is stable in the absence of surfactants. Ionic detergents can inhibit the reaction of IgG with some antigens, but these agents have little effect on the ability of IgY to bind antigens. IgY is monomeric in 0.15 M NaCl, and is dimeric in 1.5 M NaCl, while IgG is monomeric at both low and high salt conditions. The properties of IgY are described in detail in US Patent No. 4550019. The structural differences mean that the hinge region which is present in IgG between the Fab pieces is absent in IgY. This hinge region renders IgG less stable than IgY, and hence IgG is slightly less suitable than IgY for use in solid-phase extraction procedures.

The yolk of eggs laid by immunized chickens is an abundant source of polyclonal antibodies (pAb). Specific antibodies produced in chickens offer several important advantages over producing antibodies in other mammals, such as those mentioned above.

Due to the phylogenetic distance between birds and mammals, there is greater probability of producing a higher percentage of specific antibody against mammalian

antigens when using chickens. Highly conserved mammalian proteins sometimes fail to elicit a humoral immune response in animals, such as rabbits, which are traditionally used for generating polyclonal antibody. Since chicken IgY does not cross-react with mammalian IgG, and does not bind bacterial or mammalian Fc receptors, non-specific binding is reduced, and the need for cross-species immunoabsorptions is also eliminated.

Two affinity matrices were used in the pre-fractionation of serum or plasma for 2DE and the consequent display of the low abundance proteome. Affi-gel Blue has been previously used to remove albumin from samples, with depletion of certain other proteins. However, to our knowledge it has not hitherto been suggested that Affi-gel Blue could be useful in preparation of samples for 2DE analysis. Affi-gel Blue and similar supports, such as HiTrap Blue P (Amersham Biosciences), are agarose supports coupled to the dye Cibacron Blue F3G-A, which has a high affinity for albumin, as well as for interferon, a broad range of nucleotide-requiring enzymes, α_2-macroglobulin, coagulation factors, and nucleic acid-binding proteins. Thus it depletes not only albumin but also α_2-macroglobulin and coagulation factors from plasma.

Example 1 Production of Polyclonal Antibodies to Human Plasma

First generation polyclonal antibodies to human plasma were produced in female chickens. The procedure is summarised in Figure 2. Chickens (14 week old White Leghorn / Rhode Island Red cross) were immunised according to the recommendations of the 21st European Centre for the Validation of Alternative Methods (ECVAM) workshop, using 1 mg plasma proteins/bird (12.5 µl of 80 mg/ml) suspended in saline (87.5 µl)/Freund's Incomplete Adjuvant (100 µl). 100 µl of total plasma proteins were injected

subcutaneously over the pectoralis major muscle, using a 25-gauge needle at four sites (i.e. 50 µl per site).

Birds received three booster injections as described above, 4, 8 and 12 weeks later. Eggs were collected prior to immunization and the yolks stored at -20°C. Eggs were collected daily during the immunization schedule, up to 30 days after the last booster injection and the yolks extracted as described in Example 2.

Example 2 Extraction of IgY

Egg yolks (10 per batch) were separated and then suspended in 2 volumes of 100 mM phosphate buffer (pH7.6) in a glass beaker. An equal volume of chloroform was added and then stirred for 5 min at room temperature. The resultant emulsion was then transferred to 100 ml glass centrifuge tubes and centrifuged at 2000 g for 1 h at 4°C. The supernatant was collected and its volume determined. PEG 6000 (Sigma Chemical Company, St Louis, USA) was dissolved in the supernatant to final concentration of 12% w/v, incubated for 10min at room temperature and then centrifuged at 2000g for 1h at 4°C. The supernatant was discarded and the pellet resuspended in 100 mM phosphate buffer pH7.6 (1/6 original yolk volume) and stored at -20°C as 1 ml aliquots.

Egg yolks were collected for four weeks following the final immunization, pooled and extracted as described above.

The binding characteristics of the extracted antibodies were determined by 2DE Western Blot analysis as described below. The antibodies were then coupled to Sepharose 4B according to the manufacturer's instructions.

Example 3 Coupling of IgY to Sepharose 4B

PEG 6000 was dissolved in 2ml IgY solution (17.3mg protein/ml), incubated for 10 min at room temperature and then centrifuged at 2000g for 1h. The pellet was resuspended in coupling buffer (0.1M $NaHCO_3$ pH

8.3, containing 0.5M NaCl) to a final concentration of 7.5 mg protein/ml.

CNBr-activated Sepharose 4B (Pharmacia; 1 g) was suspended in 20 ml of 1 mM HCl. The suspension was then washed with 200 ml 1mM HCl on a sintered glass filter. The washed gel was resuspended in the IgY solution, and mixed on a rotary mixer for 18h at 4°C. The gel was then washed with 5 volumes of coupling buffer and incubated in 0.1M Tris-HCl buffer, pH8.0 for 2h at 4°C. The gel was washed 3 times alternately with 5 volumes 0.1M acetate buffer pH 4.0 containing 0.5M NaCl, and then 0.1M Tris HCl pH 8.0 containing 0.5M NaCl. The anti-human plasma antibody-Sepharose 4B (AHP-Sepharose) gel was then stored at 4°C in 0.01 M phosphate-buffered saline, pH7.4, containing 0.05% sodium azide as a preservative.

Example 4 Preparation Of Affinity-depleted and Immunodepleted Plasma

Plasma from normal human males was subjected to affinity depletion using Affi-gel Blue and immunodepletion using AHP-Sepharose. Affi-gel Blue (5ml gel suspension per ml of plasma) was suspended in sealed 10ml polypropylene columns (Econo-Columns; Bio-Rad) and eluted with 2 volumes of 20mM phosphate buffer (pH7.1). Plasma (500 µl) was mixed with an equal volume of 20 mM phosphate buffer (pH7.1) and mixed on a rotary mixer for 4h at 4°C ml. This solution was then added to the Affi-gel Blue column. The column was capped and mixed on a rotary mixer for 18h at 4°C. The column tip seal and cap were removed, and the flow-through collected. The protein content was determined, and the aliquot was stored at -80°C for subsequent analysis.

The residual Affi-gel-treated plasma was then subjected to AHP-Sepharose immunodepletion as follows. AHP-Sepharose (100 µl) was washed with 4 volumes of 100mM phosphate buffer (pH7.1) using a sintered glass filter. The washed gel was resuspended in Affi-gel Blue-treated

plasma (100 µl) in a 2ml microcentrifuge tube, and mixed on a rotary mixer for 18h at 4°C. The suspension was centrifuged at 13,200 g for 5 min at room temperature and the supernatant collected, its protein content determined and the aliquot then stored at -80°C for subsequent analysis.

Affinity-depleted and immunodepleted plasma was then used as antigen to raise second-generation antibodies in chickens, using the same immunization schedule as in Example 1. The antibodies raised were evaluated individually and pooled for evaluation of their effects on the removal of proteins from untreated and Affi-gel Blue-treated plasma respectively. The process of antibody preparation is summarised in Figure 2.

Example 5 Removal of High Abundance Proteins from Human Serum Samples

Human serum samples were treated with Affi-gel Blue for the primary removal of albumin by the following process. For serum samples, whole blood (2ml) was collected by venepuncture into plain collection tubes, in which blood was allowed to clot at room temperature for 30 min and then processed. Samples were then centrifuged at 2000g for 10 min, after which serum was collected. For plasma samples, whole blood was collected in the same way into EDTA anticoagulant tubes. An aliquot (100 µl) was removed for the determination of total protein. Serum and plasma samples were stored at -80°C until analysed.

Samples were thawed at room temperature and incubated with 5 volumes of Affi-gel Blue for 16h at 4°C room on a rotary platform. Samples were then centrifuged at 2000g for 10 min. The supernatant was recovered, an aliquot (100 µl) was removed for the determination of total protein and 2DE analysis, and the remainder of the sample was incubated with anti-human plasma antibody coupled to Sepharose 4B for 4h at 4°C. The samples were then centrifuged for 20 min at 2000g at 4°C. The

supernatant was recovered, an aliquot (50 µl) was removed for determination of total protein, and the remainder stored at -80°C until subjected to 2DE analysis.

Example 6 Two-Dimensional Electrophoresis

First Dimension Separation

2 µl serum diluted in 48 µl of sample preparation buffer (62.5 mM Tris HCl, 2% SDS, 25% glycerol, 0.01% bromophenol blue and 2.3 % DTT, pH 7.8), and incubated at 95°C for 5 min. 15 µg (~7 µl) of serum protein was mixed with solubilization buffer (7M urea, 2M thiourea, 100 mM DTT, 4% CHAPS, 0.5% carrier ampholytes pH4-7, 0.01% BPB and 40mM Tris) to a final volume of 200 µl and incubated for 1h at room temperature. This mixture was then applied to a Ready Strip (11 cm, pH 4-7, Bio-Rad) and actively rehydrated at 50V and 20°C for 16h. Serum proteins were isoelectrically focused at 250V for 15 min and then 8000V for 150 min, and then maintained at 8000V for a total of 35000Vh/gel, i.e. a total of 42000 Vh per gel. Ready Strips were then stored at -80°C until second dimension processing.

Second Dimension Separation

Ready Strips from the first dimension separation were equilibrated in 6 ml of equilibration buffer (50mM Tris-HCl pH 8.8, 6M urea, 30% glycerol, 2% SDS, BPB, 5mM TBP). Strips were rinsed in Tris-glycine SDS running buffer (25mM Tris, 192 mM glycine, 0.% w/v SD, pH 8.3) and then applied to the top of a Ready Gel (10% or 8-16% acrylamide, Criterion Gel; Bio-Rad). Low melting point agarose (0.5% in running buffer containing BPB) was layered on top of the strip. Two wicks to which molecular weight markers were applied were inserted into the agarose. Gels were electrophoresed at 10mA/gel for 1hr, 20mA/gel for 2h and then at 30mA/gel for 30 min. Gels were then fixed in methanol/acetic acid (40%/10% in deionised MilliQ water for 1h at room temperature and then

incubated in Sypro Ruby® (Bio-Rad) for 16h at room temperature on a rocking platform. Gels were destained for 1 h in methanol/acetic acid (10%/7% in dH_2O). The gels were imaged using a Bio-Rad FX imager at 100 nM resolution.

Figure 4 shows a comparison between the number of protein species identifiable by 2DE which can be detected using pooled

a) first and second round anti-human plasma chicken antiserum in control, untreated human plasma,
b) plasma subjected to plasma subjected to affinity depletion and immunodepletion using first generation polyclonal antibody, and
c) plasma subjected to affinity depletion and immunodepletion using second generation polyclonal antibody.

The analysis of the protein spots in Figure 4 is summarised in Table 1. This shows that the method of this invention leads to the removal of the majority of the protein spots present in undiluted, untreated control plasma (neat plasma), while revealing a very high proportion of previously undetected proteins.

TABLE 1

Comparison of protein spots identified by image analysis of 2DE display of untreated serum (neat), serum treated with Affi-gel blue (AGB) and serum treated with AHP-Sepharose.

PROTEIN SPOT COMPARISION	neat (untreated)	AGB Treated	AHP Sepharose Treated
Total number of protein spots displayed	248	152	157
Number of spots common to both neat and post-treatment protein displays	NA	127	130
Number of spots identified only after treatment	NA	25	27
Number of protein spots increased by 5-fold or greater following treatment compared to neat	NA	9	9
Number of protein spots decreased by 5-fold or greater following treatment compared to neat	NA	28	16
Spot unique to specific treatment (AGB vs AHP)	NA	14	15
Number of protein spots that were ≥ 2-fold greater in AGB than AHP Sepharose	NA	12	-
Number of protein spots that were < 2-fold less in AGB than AHP Sepharose	NA	20	-

NA : not applicable

Example 7 Device for Rapid Sample Processing

The process and product of the invention for the preparation of either serum or plasma samples for the display of a low abundance proteome may be used in the form of a sealed Luer-type cartridge suitable for use together with plastic syringes. Anti-human plasma antibody resin (0.5ml) is sandwiched between two bands (0.5ml each) of Affi-gel Blue resin, or other protein-binding resin, in a 1.5 ml cartridge. A 2.5ml syringe containing 1ml of serum is connected to one end of the cartridge, and an empty 2.5 ml syringe is connected to the other end of the cartridge. This device is illustrated in Figure 6. The serum sample is refluxed through the cartridge 5 times, and then collected and stored for 2DE analysis. The cartridge and syringe may be provided as a kit.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

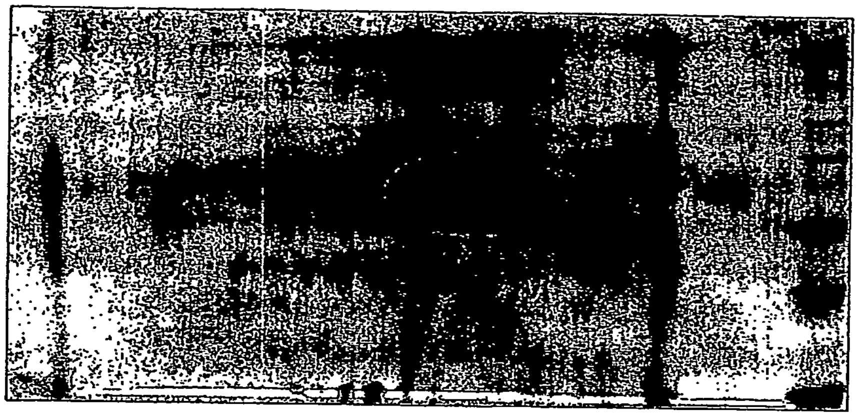

Figure 1

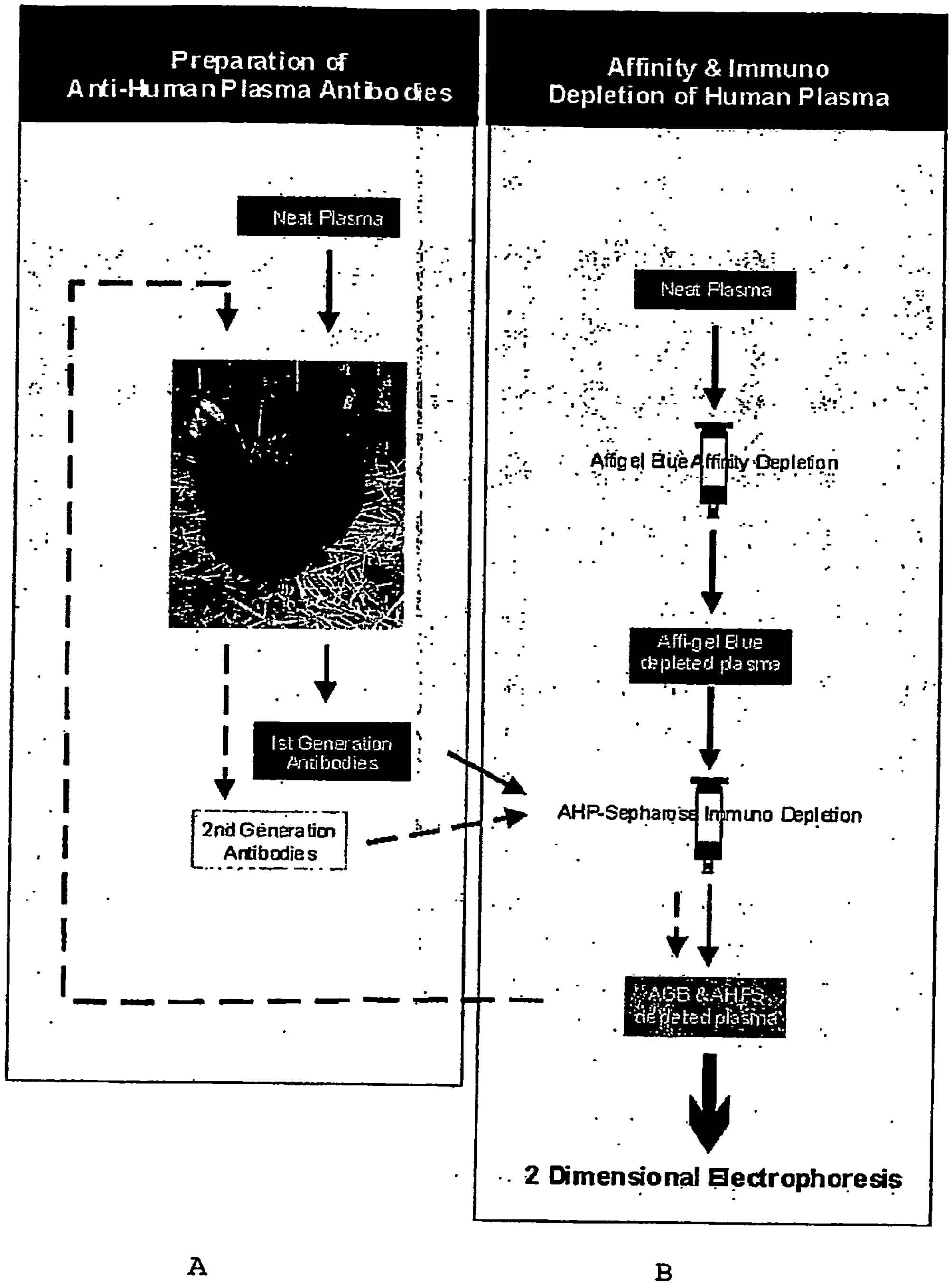
Preparation of Anti-Human Plasma Antibodies
Neat Plasma
Ist Generation Antibodies
2nd Generation Antibodies
Affinity & Immuno Depletion of Human Plasma
Neat Plasma
Affigel Blue Affinity Depletion
Affi-gel Blue depleted plasma
AHP-Sepharose Immuno Depletion
AGB & AHPS depleted plasma
2 Dimensional Electrophoresis
A
B

Figure 2

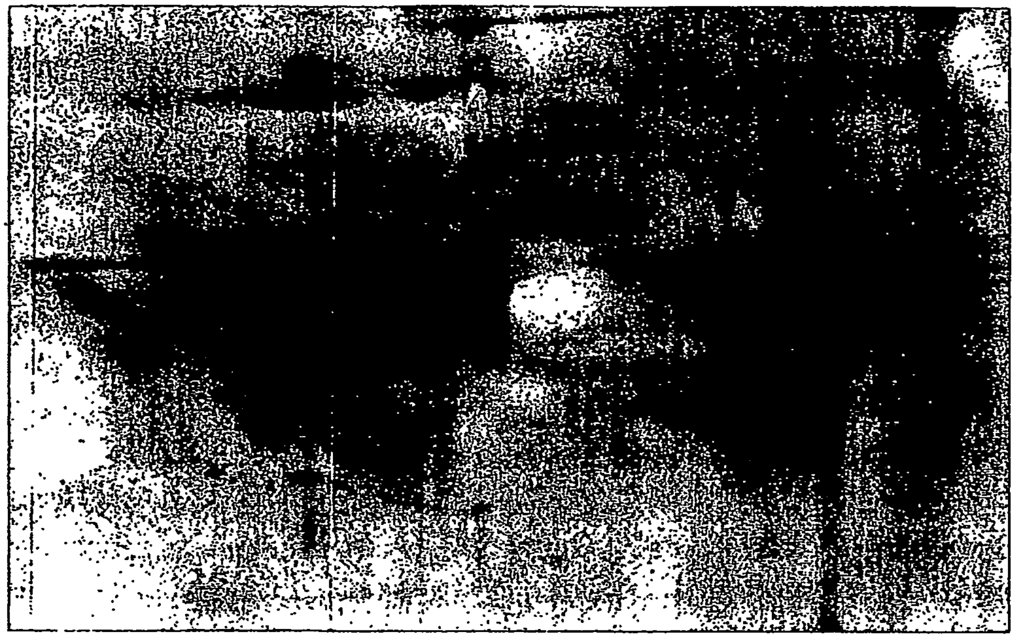

Figure 3

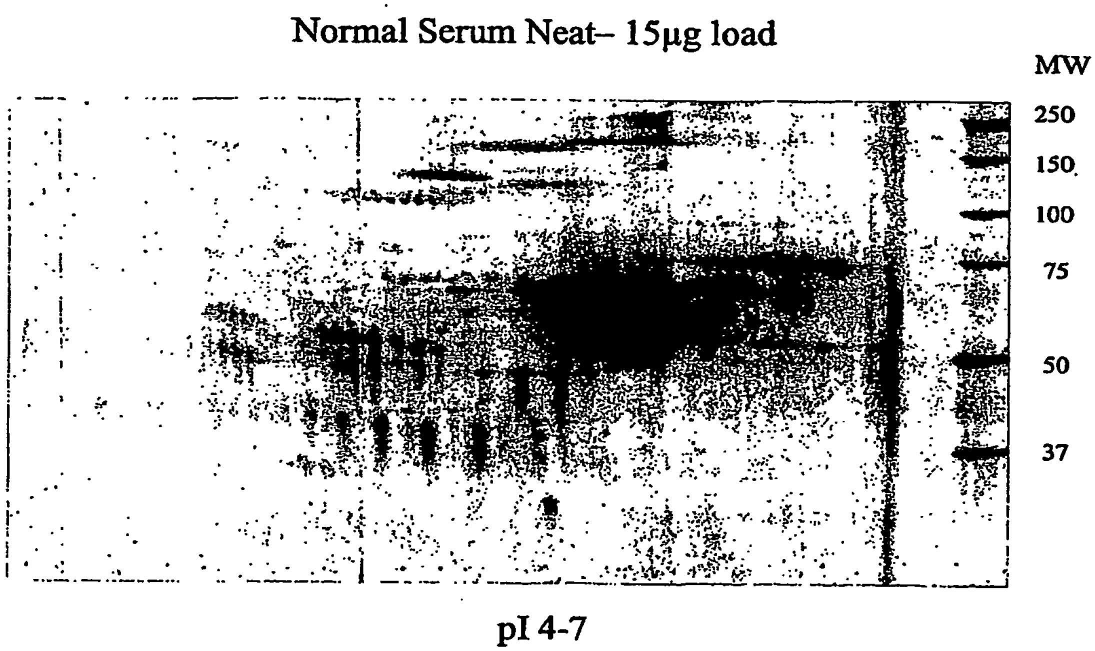

Figure 4A

Normal Serum Affi-Gel Blue Gel Treated 25µg load

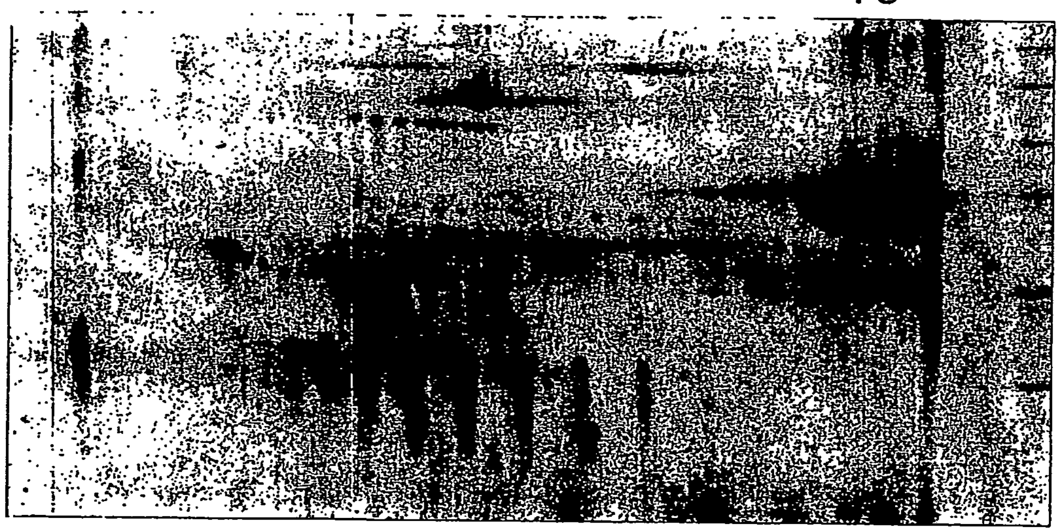

Figure 4B

Normal Serum AGB & Sepharose-IgY Treated – 25µg load

Figure 4C

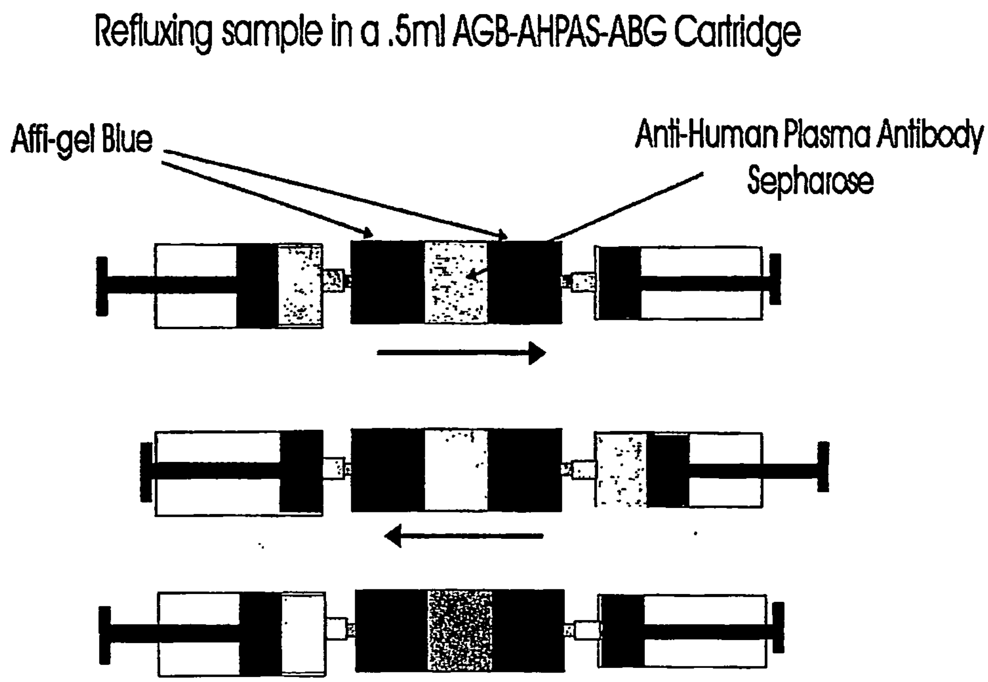
Refluxing sample in a .5ml AGB-AHPAS-ABG Cartridge
Affi-gel Blue
Anti-Human Plasma Antibody
Sepharose

Figure 5